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Sequential stimulation of rat cerebellar granular layer in vivo: Further evidence of a ‘tidal-wave’ timing mechanism in the cerebellum

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Abstract

Here we present evidence that the cerebellar cortex in vivo specifically responds to sequential input to the granular layer, the main input site of the cerebellar cortex. Ordered sequences of electrical stimuli were delivered through an array of stimulating electrodes in such a way, that an apparent movement of the stimulus was produced. The parallel fiber population responses to sequential stimuli ‘moving’ at 7 different velocities (0.1–0.7 m/s) and in two different directions (towards and away from the recording site) were measured extracellularly in the molecular layer. Population responses were maximal when the stimulus moved towards the recording site at a velocity close to the conduction velocity of parallel fibers. Responses were significantly reduced when the stimulus velocity was higher or lower. We conclude that the characteristic geometrical arrangement of parallel fibers enables the cerebellum to specifically detect precise spatio-temporal activity patterns in the mossy fiber system. These findings confirm earlier observations made in vitro and shed new light on the functional interpretation of cerebellar anatomy. Together with recent findings suggesting that precise spatio-temporal activity patterns play a key role in information processing in the neocortex, the results reported here are particularly important concerning the information exchange between the strongly interconnected cerebellum and neocortex. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cerebellar cortex; Parallel fibers; Spatio-temporal activity patterns; Tidal-wave theory

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1. Methods

Experiments were performed in three Sprague-Dawley rats weighing 200–300 g. Animals were anesthetized with an initial dose of Ketamine (80 mg/kg body weight) and Xylazine (20 mg/kg body weight) and anesthesia was maintained with small doses of Ketamine (ca. 30 mg/kg body weight) as needed. All animal experiments were carried out in accordance with the national and institutional guidelines on the use of animals in research.

After reaching surgical depth of anesthesia the animals were fixated in a stereotaxic frame and folia VI–VIII of the cerebellar vermis were exposed. The dura was removed and the tissue was covered with mineral oil. Recordings of parallel fiber population activity were performed using Teflon insulated tungsten microelectrodes with 12 MΩ impedance (AM Systems, USA) inserted in the molecular layer. An array of stimulating electrodes was built as described previously [8,9]. Electrodes identical to those used for our recordings (Teflon insulated tungsten, 12 MΩ) were glued together with cyanoacryl to form a linear array of 5 (in one experiment only 3) stimulating electrodes with their tips separated by 140 μm. This array was slowly inserted into the cerebellum while using the center electrode for spike activity recording in order to determine the depth of penetration: the onset and fading of its characteristic high frequency spiking activity indicated the passage of the electrode tip through the Purkinje cell layer into the granular layer. All recordings were performed in the superficial vermal folia, i.e., those folia which permitted the visual inspection of stimulating and recording electrodes by a surgical stereomicroscope. As a control, in one experiment the stimulated sites were lesioned by injection of a DC current (15 μA for 1 s at 1.5 Hz for 5 s) through the electrodes and the location of electrode tips in the granular layer was anatomically verified. After the final tip position was reached, the center electrode was disconnected from the amplifier and connected to a stimulus isolation unit (SI-A360, WPI), as were the remaining stimulating electrodes. Alignment of the stimulating electrodes with the recording site along the parallel fiber beam was tested by stimulating through the farthest stimulating electrode while their tips were still in the molecular layer.

Sequential stimuli simulating movement of the stimulus through the array of electrodes were produced as described in detail elsewhere [8,9]. In short, stimulus current was adjusted for each electrode individually to produce a parallel fiber population response to a single stimulus in the range of 100–200 μV typically resulting in stimulation currents in the range between 50 and 250 μA. Sequential or 'moving' stimuli were generated by stimulation through electrode 1–5 using the same delay between any two neighboring stimulus sites. Thus, the velocity of the 'movement' was given by the delay between individual stimuli, and movement direction by the sequence (1–2–3–4–5) vs. (5–4–3–2–1). When electrode 1 was the closest to the recording site, the sequence 1–2–3–4–5 produced an apparent movement away from, and the inverse sequence 5–4–3–2–1 produced a movement towards the recording site (cf. Fig. 1). We measured parallel fiber population responses to stimuli moving in both directions at 7 different velocities (0.1–0.7 m/s, in steps of 0.1 m/s).

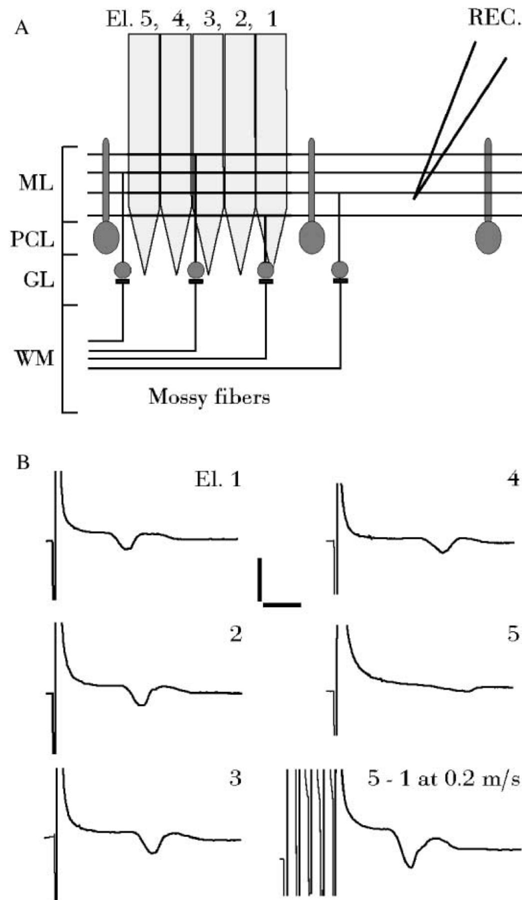

Fig. 1. Experimental arrangement and results of single and sequential stimulation. (A) Scheme of the arrangement of stimulating electrodes (El. 1–5) and the recording electrode (REC.) in the cerebellar cortex as seen in the transversal plane. An array of five stimulating electrodes was lowered into the granular layer, where the main input to the cerebellum, the excitatory mossy fibers terminate and form synapses with granular cells. Their axon ascends through the Purkinje cell layer into the molecular layer and bifurcates to form the two branches of a single parallel fiber. Purkinje cells, with their cell bodies located in the Purkinje cell layer and with their flat dendritic tree in the molecular layer, receive about 200.000 synaptic inputs from the same number of different parallel fibers. The recording electrode was placed in the molecular layer aligned with the array of stimulating electrodes along the beam of parallel fibers. (B) Extracellularly recorded responses to stimulation with each single electrode alone (El. 1–5). Parallel fiber population response appears as a short negative deflection 3–8 ms after stimulation. Note the systematic increase in stimulus-response delay with increasing distance between stimulating electrode and recording site reflecting the conduction velocity in parallel fibers. Graph at lower right: response to a sequence of stimuli (5–4–3–2–1) timed to simulate movement of the stimulus at 0.2 m/s towards the recording site. The sequential stimulation produced five electrical artifacts and a single parallel fiber population response which, for velocities of apparent stimulus movements close to that of spike conduction in parallel fibers, was larger than any of the responses to a single stimulus. All traces shown are averages over 30 trials. Traces for the individual responses (El. 1–5) are aligned on the single electrical stimulus artifact and for the sequential stimulus on the last of the series of five artifacts. ML molecular layer, PCL Purkinje cell layer, GL granular layer, WM white matter. Scale bars are 1 mV and 2 ms.

2. Results

Sequential stimulation of the cerebellar granular layer with a 'moving' stimulus lead to a velocity and direction dependent parallel fiber population response (Fig. 2). The maximal response amplitude occurred at velocities of the stimulus of 0.2 (2 animals) and 0.3 m/s (1 animal). For velocities above and below this value response amplitudes were significantly reduced with the smallest amplitude typically occurring at 0.1 m/s (Fig. 2). In a similar experiment performed in acute slices of rat cerebellum in vitro [7–10] the response amplitudes were close to zero for very high or low velocities. In those experiments, however, the stimulus current was chosen such that stimulation with a single electrode did not produce any visible population spike, while in the present experiments the stimulus current evoked a population response in each individual electrode (Fig. 1B).

The stimulus 'velocity' which elicited a maximal response amplitude in our experiments is lower than the conduction velocity in parallel fibers which was found to be

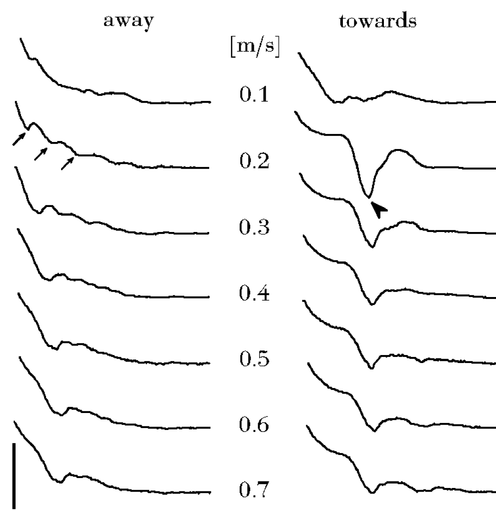

Fig. 2. Parallel fiber population responses to stimulation with sequential patterns producing an apparent movement of the stimulus away from (left) or towards (right) the recording site at 7 different velocities (0.1–0.7 m/s). Each trace represents an average over 30 trials. Parallel fiber population spikes occur as short negative deflections. The response was maximal for movement of the stimulus towards the recording site at a velocity of 0.2 m/s (arrowhead). At low velocities several small negative deflections occur (examples marked by arrows in the 0.2 m/s trace on the left), corresponding to the effects of the individual stimuli. With increasing velocity, response patterns become increasingly similar and less dependent on velocity. The higher the velocity, i.e. the shorter the inter-stimulus interval the more resemble the stimuli a synchronous stimulation. For stimuli moving away from the recoding site the first response peak shifts to the right with increasing velocity. This is because the beginning of the traces were aligned on the last stimulus artifact and since with increasing velocity the total stimulus artifact becomes shorter in duration we have an apparent shift of the first response peak to the right. If the traces were aligned on the first stimulus artifact the first response peak would always appear at a constant delay. Scale bars are 1 mV and 2 ms.

somewhat higher between 0.3 and 0.4 m/s [5,6] in warm-blooded animals. Since we recorded and stimulated at the exposed surface of the cerebellar cortex the conduction velocity was most likely reduced due to a drop in temperature at the surface.

3. Discussion

We have shown here that the cerebellar cortex in vivo shows specific responses to sequential stimulation of its main input site, the granular layer. These experiments confirm results obtained earlier in acute slice preparations of the cerebellar cortex in vitro. Acute slices, in spite of their general usefulness, differ from the in vivo situation among other things because of the absence of permanent background activity. The different network behavior in slices may be partly due to the lack of afferences and of intrinsic connections severed by the slicing.

The findings presented here together with other theoretical [2,3] and experimental [4,7–10] work shed new light on the functional interpretation of cerebellar anatomy. The assumption that precise spatio-temporal patterns are relevant in the input to the cerebellum gives a conclusive explanation for the characteristic cerebellar anatomy. Most interestingly, it is also congruent with recent experimental findings obtained in the neocortex, showing that precise spatio-temporal activity patterns instead of or besides spike rate coding, might be the basis of information processing in the neocortical network [1,11,12]. We suggest that the cerebellar cortex, through the geometrical arrangement of the parallel fibers and Purkinje cells, is designed to specifically detect spatio-temporal aspects, (i.e. sequential, apparently 'moving' input) in the mossy fiber activity and therefore also in the cortico-cerebellar input. Thus, dynamic rather than static or sustained aspects of mossy fiber activity would drive the cerebellum to deliver its contribution to motor performance.

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References

[1] M. Abeles, H. Bergman, E. Margalit, E. Vaadia, Spatiotemporal firing patterns in the frontal cortex of behaving monkeys, J. Neurophysiol. 70 (1993) 1629–1638.

[2] V. Braitenberg, The cerebellum revisited, J. Theoret. Neurobiol. 2 (1983) 237–241.

[3] V. Braitenberg, R.P. Atwood, Morphological observations on the cerebellar cortex, J. Comput. Neurol. 109 (1958) 1–33.

[4] V. Braitenberg, D. Heck, F. Sultan, The detection and generation of sequences as a key to cerebellar function. Experiments and theory, Behav. Brain Sci. 20 (1997) 229–245.

[5] R.S. Dow, Action potentials of cerebellar cortex in response to local electrical stimulation, J. Neurophysiol. 12 (1949) 245–256.

[6] J.C. Eccles, R. Llinás, K. Sasaki, Parallel fibre stimulation and the responses induced thereby in the Purkinje cells of the cerebellum, Exp. Brain Res. 1 (1966) 17–39.

[7] D. Heck, Rat cerebellar cortex in vitro responds specifically to moving stimuli, Neurosci. Lett. 157 (1993) 95–98.

[8] D. Heck, Investigating dynamic aspects of brain function in slice preparations: Spatiotemporal stimulus patterns generated with an easy to build multi-electrode array, J. Neurosci. Methods 58 (1995) 81–87.

[9] D. Heck, Sequential input to guinea pig cerebellar cortex in vitro strongly affects Purkinje cells via parallel fibers, Naturwissenschaften 82 (1995) 201–203.

[10] D. Heck, Sequential stimulation of rat and guinea pig cerebellar granular cells in vitro leads to increasing population activity in parallel fibers, Neurosci. Lett. 263 (1999) 137–140.

[11] A. Riehle, S. Grün, M. Diesmann, A. Aertsen, Spike synchronization and rate modulation differentially involved in motor cortical function, Science 278 (1997) 1950–1953.

[12] B.L. Strehler, R. Lestienne, Evidence on precise time-coded symbols and memory of patterns in monkey cortical neuronal spike trains, Proc. Natl. Acad. Sci. 83 (1986) 9812–9816.